

Amendments to the Claims

This listing of claims will replace all prior versions, and listings of claims in the application.

1-17. (cancelled)

18. (new) A method for identifying a translational fusion partner (TFP) for secretion of a target protein, the method comprising:

a) preparing an automatic screening vector comprising a polynucleotide encoding said target protein linked in frame to a polynucleotide encoding a reporter protein;

b) linking a plurality of polynucleotide fragments to said automatic screening vector to create a library;

c) transforming said library into cells having no activity of said reporter protein;

d) culturing said cells; and

e) identifying a TFP by detecting reporter protein activity secreted from one or more of said cells.

19. (new) The method of claim 18, further comprising a step of isolating the identified TFP.

20. (new) The method of claim 18, wherein said target protein is selected from the group consisting of cytokines, serum proteins, immunoglobulins, cytokine receptors, lactoferrin, interferons, colony stimulating factors, stem cell factor, phospholipase activating

protein, insulin, tumor necrosis factor, growth factors, hormones, enzymes, anticancer peptides, and antibiotic peptides.

21. (new) The method of claim 18, wherein said target protein is a non-producible protein.

22. (new) The method of claim 18, wherein said target protein is human interleukin-2, human granulocyte colony stimulating factor, or CalB14.

23. (new) The method of claim 18, wherein said plurality of polynucleotide fragments is from genomic DNA.

24. (new) The method of claim 18, wherein said plurality of polynucleotide fragments is from cDNA.

25. (new) The method of claim 18, wherein said plurality of polynucleotide fragments is from animal, plant, or microorganism DNA.

26. (new) The method of claim 25, wherein said plurality of polynucleotide fragments is from yeast DNA.

27. (new) The method of claim 25, wherein said plurality of polynucleotide fragments is from *Candida*, *Debaryomyces*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Schizosaccharomyces*, *Yarrowia*, *Saccharomyces*, *Aspergillus*, *Penicillium*, *Rhizopus*, or *Trichoderma* DNA.

28. (new) The method of claim 18, wherein said cell is a eucaryotic or bacterial cell.

29. (new) The method of claim 28, wherein said cell is an *Escherichia*, *Pseudomonas*, *Bacillus*, *Streptomyces*, *Spodoptera frugiperda*, CHO, COS 1, COS 7, BSC 1, BSC 40, BMT 10, *Candida*, *Debaryomyces*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Schizosaccharomyces*, *Yarrowia*, *Saccharomyces*, *Aspergillus*, *Penicillium*, *Rhizopus*, or *Trichoderma* cell.

30. (new) The method of claim 18, wherein said reporter protein is an extracellularly secreted protein.

31. (new) The method of claim 30, wherein said reporter protein is selected from the group consisting of invertase, sucrase, cellulase, xylanase, maltase, amylase, glucoamylase, and galactosidase.

32. (new) The method of claim 31, wherein said reporter protein is invertase and said cell is cultured on medium containing only sucrose as a carbon source.

33. (new) The method of claim 18, wherein said automatic screening vector further comprises a promoter.

34. (new) The method of claim 33, wherein said promoter is from a gene selected from the group consisting of GAPDH, PGK, ADH, PHO5, GAL1, and GAL10.

35. (new) The method of claim 18, wherein said automatic screening vector further comprises a cleavage recognition site.

36. (new) The method of claim 35, wherein said cleavage recognition site is recognized by Kex2p.

37. (new) The method of claim 18, wherein said automatic screening vector comprises a promoter, a polynucleotide encoding a target protein, which is deleted for translation initiation and termination codons, and a polynucleotide encoding a reporter protein fused in frame to the polynucleotide encoding the target protein.

38. (new) A method for identifying a TFP for secretion of a target protein, the method comprising:

a) preparing an automatic screening vector comprising a polynucleotide encoding said target protein linked in frame to a polynucleotide encoding invertase;

b) linking a plurality of polynucleotide fragments to said automatic screening vector to create a library;

c) transforming said library into a yeast mutant strain deleted for its endogenous invertase gene;

d) culturing said transformed yeast on a medium containing only sucrose as a carbon source; and

e) identifying a TFP by detecting invertase activity secreted from one or more of said transformed yeast.

39. (new) A method of producing a TFP library, the method comprising:

a) preparing an automatic screening vector comprising a polynucleotide encoding said target protein linked in frame to a polynucleotide encoding a reporter protein;

b) linking a plurality of polynucleotide fragments to said automatic screening vector to create a library;

c) transforming said library into cells having no activity of said reporter protein;

d) culturing said cells;

e) identifying cells secreting reporter protein activity; and

f) collecting said cells secreting reporter protein activity;

thereby producing a TFP library.

40. (new) The method of claim 39, further comprising the step of isolating a polynucleotide encoding said TFP from each collected cell.

41. (new) The method of claim 39, wherein said target protein is selected from the group consisting of cytokines, serum proteins, immunoglobulins, cytokine receptors, lactoferrin, interferons, colony stimulating factors, stem cell factor, phospholipase activating protein, insulin, tumor necrosis factor, growth factors, hormones, enzymes, anticancer peptides, and antibiotic peptides.

42. (new) The method of claim 39, wherein said target protein is a non-producible protein.

43. (new) The method of claim 42, wherein said target protein is human interleukin-2, human granulocyte colony stimulating factor, or CalB14.

44. (new) The method of claim 39, wherein said plurality of polynucleotide fragments is from genomic DNA.

45. (new) The method of claim 39, wherein said plurality of polynucleotide fragments is from cDNA.

46. (new) The method of claim 39, wherein said plurality of polynucleotide fragments is from animal, plant, or microorganism DNA.

47. (new) The method of claim 46, wherein said plurality of polynucleotide fragments is from yeast DNA.

48. (new) The method of claim 46, wherein said plurality of polynucleotide fragments is from *Candida*, *Debaryomyces*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Schizosaccharomyces*, *Yarrowia*, *Saccharomyces*, *Aspergillus*, *Penicillium*, *Rhizopus*, or *Trichoderma* DNA.

49. (new) The method of claim 39, wherein said cell is a eucaryotic or bacterial cell.

50. (new) The method of claim 49, wherein said cell is an *Escherichia*, *Pseudomonas*, *Bacillus*, *Streptomyces*, *Spodoptera frugiperda*, CHO, COS 1, COS 7, BSC 1, BSC 40, BMT 10, *Candida*, *Debaryomyces*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Schizosaccharomyces*, *Yarrowia*, *Saccharomyces*, *Aspergillus*, *Penicillium*, *Rhizopus*, or *Trichoderma* cell.

51. (new) The method of claim 39, wherein said reporter protein is an extracellularly secreted protein.

52. (new) The method of claim 51, wherein said reporter protein is selected from the group consisting of invertase, sucrase, cellulase, xylanase, maltase, amylase, glucoamylase, and galactosidase.

53. (new) The method of claim 52, wherein said reporter protein is invertase and said cell is cultured on medium containing only sucrose as a carbon source.

54. (new) The method of claim 39, wherein said automatic screening vector further comprises a promoter.

55. (new) The method of claim 54, wherein said promoter is from a gene selected from the group consisting of GAPDH, PGK, ADH, PHO5, GAL1, and GAL10.

56. (new) The method of claim 39, wherein said automatic screening vector further comprises a cleavage recognition site.

57. (new) The method of claim 56, wherein said cleavage recognition site is recognized by Kex2p.

58. (new) The method of claim 39, wherein said automatic screening vector comprises a promoter, a polynucleotide encoding a target protein, which is deleted for

translation initiation and termination codons, and a polynucleotide encoding a reporter protein fused in frame to the polynucleotide encoding the target protein.

59. (new) A TFP library produced by the method of claim 39.

60. (new) A TFP identified by the method of claim 18 or an analogue thereof having at least 75% amino acid homology to the amino acid sequence of said TFP or a fragment of said TFP, wherein said TFP or an analogue or fragment thereof induces secretion of a target protein.

61. (new) The TFP of claim 60, comprising the amino acid sequence of one of SEQ ID NOS:1, 3, 5, 7, 9, 40, or 42, or an analogue thereof having at least 75% amino acid homology to the amino acid sequence of said TFP or a fragment of said TFP, wherein said TFP or an analogue or fragment thereof induces secretion of a target protein.

62. (new) A polynucleotide encoding the TFP of claim 60 or an analogue thereof having at least 75% nucleotide homology to the polynucleotide or a fragment of said polynucleotide, wherein said polynucleotide or an analogue thereof or a fragment thereof encodes a TFP that induces secretion of a target protein.

63. (new) The polynucleotide of claim 62, wherein said polynucleotide encodes a TFP comprising the amino acid sequence of one of SEQ ID NOS:1, 3, 5, 7, 9, 40, or 42, or an analogue thereof having at least 75% amino acid homology to the amino acid sequence of said TFP or a fragment of said TFP, wherein said TFP or an analogue or fragment thereof induces secretion of a target protein.

64. (new) The polynucleotide of claim 62, wherein said polynucleotide comprises the nucleotide sequence of one of SEQ ID NOS:2, 4, 6, 8, 10, 41, or 43 or an analogue thereof having at least 75% nucleotide homology to the polynucleotide or a fragment of said polynucleotide.

65. (new) A vector comprising the polynucleotide of claim 62.

66. (new) The vector of claim 65, wherein said vector is selected from the group consisting of pYIL-KRTFP1 (KCTC 10544BP), pYIL-KRTFP2 (KCTC 10545BP), pYIL-KRTFP3 (KCTC 10546BP), pYIL-KRTFP4 (KCTC 10547BP), pYIL-KR1-3 (KCTC 10548BP), pYIL-KR1-4 (KCTC 10549BP), pYGT3-1-1-GCSF (KCTC 10753BP), and pYGT3-1-2-GCSF (KCTC 10754BP).

67. (new) A cell transformed with the vector of claim 65.

68. (new) The cell of claim 67, wherein said cell is selected from the group consisting of *Escherichia*, *Pseudomonas*, *Bacillus*, *Streptomyces*, *Spodoptera frugiperda*, CHO, COS 1, COS 7, BSC 1, BSC 40, BMT 10, *Candida*, *Debaryomyces*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Schizosaccharomyces*, *Yarrowia*, *Saccharomyces*, *Aspergillus*, *Penicillium*, *Rhizopus*, and *Trichoderma*.

69. (new) A method for the recombinant production of a target protein, the method comprising:

- a) preparing an expression vector comprising a polynucleotide encoding said target protein fused to a polynucleotide encoding a TFP of claim 60;
 - b) transforming a cell with said expression vector; and
 - c) culturing said cell;
- wherein said target protein is produced.

70. (new) The method of claim 69, wherein said TFP comprises the amino acid sequence of one of SEQ ID NOS:1, 3, 5, 7, 9, 40, or 42, or an analogue thereof having at least 75% amino acid homology to the amino acid sequence of said TFP or a fragment of said TFP, wherein said TFP or an analogue or fragment thereof induces secretion of a target protein

71. (new) The method of claim 69, wherein said TFP is encoded by a polynucleotide comprising the nucleotide sequence of one of SEQ ID NOS:2, 4, 6, 8, 10, 41, or 43 or an analogue thereof having at least 75% nucleotide homology to the polynucleotide or a fragment of said polynucleotide.